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Summary and Implications

The catalytic mechanism of metmyoglobin (metMb) on the development of lipid oxidation in a phospholipid liposome model system was studied. Liposome model system was prepared with metMb solutions (2.0, 1.0, 0.5, and 0.25 mg metMb / mL) containing none, diethylenetriamine pentaacetic acid (DTPA), desferrioxamine (DFO), or ferric chloride and lipid oxidation was determined at 0, 15, 30, 60, and 90 min of incubation at 37 °C. Metmyoglobin catalyzed lipid oxidation in the liposome system, but the rate of lipid oxidation decreased as the concentration of metMb increased. The amount of free ionic iron in the liposome solution increased as the concentration of metMb increased, but the rate of metMb degradation was increased as the concentration of metMb decreased. The released free ionic iron was not involved in the lipid oxidation of model system because ferric iron has no catalytic effect without reducing agents. Both DFO and DTPA showed antioxidant effects, but DFO was more efficient than DTPA because of its multifunctional antioxidant ability as an iron and hematin chelator and an electron donor. The antioxidant activity of DTPA in liposome solution containing 0.25 mg metMb/mL was two times greater than that with 2 mg metMb/mL due to the increased prooxidant activity of DTPA-chelatable compounds. It was concluded that ferrylmyoglobin and DTPA-chelatable hematin generated from the interaction of metMb and LOOH, rather than free ionic iron, were the major catalysts in metMb-induced lipid oxidation in phospholipid liposome model system.

Introduction

It has been suggested that the interaction of metmyoglobin (metMb) with hydrogen peroxide (H_2O_2) or lipid hydroperoxides (LOOH) results in the formation of ferrylmyoglobin, which can initiate free radical chain reactions. In addition, ferrylmyoglobin as well as metMb can degrade LOOH to free radicals such as alkoxyl and peroxyl radicals, which can initiate and/or catalyze a series of propagation and termination step in the free radical chain reactions of lipid oxidation. However, others limited the role of myoglobin as only a source for free ionic iron or hematin. The ratio of peroxides to metMb is a determining factor for the formation of ferrylmyoglobin or the release of free ionic iron or hematin. Hematin is released from myoglobin in the presence of H_2O_2, followed by the liberation of free ionic iron from hematin. Hematin reacts with H_2O_2 or lipid hydroperoxide to form hematin with higher oxidation state (Ferrylhematin, Fe(IV=O)), which can initiate and propagate lipid oxidation. The concentration of metMb is a determining factor for its prooxidative activity in the presence of fatty acid or LOOH. In addition, myoglobin shows a pseudo-hydroperoxidase activity in the presence of reducing agents such as ascorbic acid and phenolic antioxidants to remove lipid hydroperoxides.

Iron chelators such as diethylenetriamine pentaacetic acid (DTPA) and desferrioxamine (DFO) have been widely used to elucidate the mechanism of iron compounds on lipid oxidation. DFO has been known as an excellent chelating agent for ferric ion and DTPA for ferrous and ferric ions. Both DFO and DTPA have chelating ability to hematin. DFO can also act as an electron donor to ferrylmyoglobin to suppress the prooxidant activity of ferrylmyoglobin and release free ionic iron from metMb as well as to free radicals to break down the free radical chain reaction of lipid oxidation.

The objectives of this study were to determine the concentration effect of metMb and the effect of ferric ion and chelators such as DFO and DTPA on the metMb-induced lipid oxidation in the phospholipid liposome model system.

Materials and Methods

The metMb-liposome model system was prepared using phospholipids. The metMb solution containing none, DTPA, DFO, or ferric chloride was added to a metMb-liposome solution and lipid oxidation was determined. In addition, the generation of nonheme iron during the incubation was measured using the ferrozine method. Lipoxygenase-like (LOX-like) activity of metMb was measured and the results were expressed as units of activity (U) per mL.

Results and Discussion

Metmyoglobin induced lipid oxidation and increased the TBARS values linearly in phospholipid liposome model system during the 90 min-incubation (Figure 1). However, the increasing rate of TBARS values significantly decreased with the increase of metMb concentration. The presence of LOOH was detected right after the preparation of the liposome model system. This result indicates that the concentration of metMb is a critical factor for determining prooxidant activity of myoglobin in the presence of LOOH and/or fatty acid.

The amount of free ionic iron significantly increased during incubation, and was proportional to the concentration of metMb (Figure 2). These results suggested that the

interaction of H_2O_2 or LOOH with metMb caused the liberation of free ionic iron as well as hematin. Thus, the LOOH preexisted or generated during the incubation should be the major catalysts to release free ionic irons from metMb because H_2O_2 was not added in this study. The addition of ferric ion did not affect myoglobin-catalyzed lipid oxidation in phospholipid liposome model system (Figure 3), indicating that either ferrylmyoglobin or hematin generated from metMb rather than free ionic iron was the major catalyst for metMb-induced lipid oxidation in this system.

Iron chelators, DTPA and DFO, showed different antioxidant effects in the liposome model system (Figure 3). DFO inhibited myoglobin-catalyzed lipid oxidation effectively, but DTPA showed only partial inhibitions. However, DFO showed stronger antioxidant activity than DTPA. The antioxidant activity of DTPA was affected by the ratio of DTPA to free ionic iron, but DFO was not. DFO can act not only as an efficient iron chelator but also an electron donor or hydrogen donor to ferrylmyoglobin, resulting in the suppression of ferrylmyoglobin-catalyzed lipid oxidation. The antioxidant activity of DTPA in liposome model system with low concentration of metMb (0.25 mg/mL) was twice as high as that with high concentration (Figures 3A and 3B), indicating that DTPA-chelatable compound was contributed more to the development of lipid oxidation at lower than at higher concentration of metMb.

LOX-like activity is related to the generation of conjugated diene at initial stage of lipid oxidation. LOX-like activity of metMb was not changed by ferric ion in the absence of reducing agents (Figure 4), indicating that free ionic ion released from myoglobin was not involved in the initiation of lipid oxidation in metMb-induced lipid oxidation. The addition of DFO and DTPA to the liposome model system decreased LOX-like activity of metMb, but DTPA suppressed it more effectively than DFO.

In this study, the catalytic mechanism of metMb on lipid oxidation was investigated in phospholipid liposome solutions incubated at 37 ºC, which is different from the refrigerated temperature conditions (4 ºC) for normal meat storage and distribution. In general, the reaction rates increase as the reaction temperature increase. Although temperature at or below 37 ºC is not likely to change the nature of metMb, it may affect the reactivity and/or solubility of metMb and other compounds such as lipids and hematin. In addition, meat products contain various anti- and prooxidative factors.

Figure 1. Lipid oxidation potential of metMb with various concentrations in phospholipid liposome model system during incubation at 37 °C for 90 min (TBARS: mmol malondialdehyde (MDA) equivalents/kg phospholipid (PL)). The concentrations of metMb in 50 mM acetate buffer (pH 5.6) were 2 (Mb2.0), 1 (Mb1.0), 0.5 (Mb0.5), and 0.25 (Mb0.25) mg per mL, respectively. Phospholipid liposome model system with buffer alone was used as a control (PL).

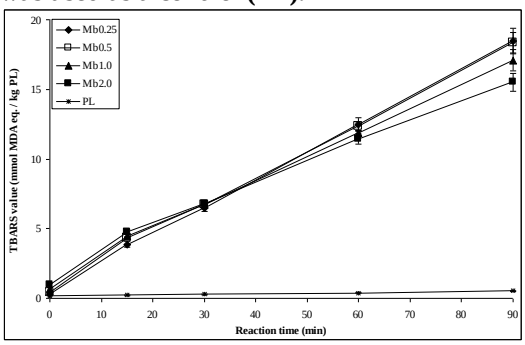


Figure 2. Formation of nonheme iron in a phospholipid liposome model system with various concentrations of metMb during incubation at 37 °C for 90 min (µg nonheme iron/mL metMb-liposome solution). The concentrations of metMb in 50 mM acetate buffer (pH 5.6) were 2 (Mb2.0), 1 (Mb1.0), 0.5 (Mb0.5), and 0.25 (Mb0.25) mg per mL, respectively. Phospholipid liposome model system with buffer alone was used as a control (PL).

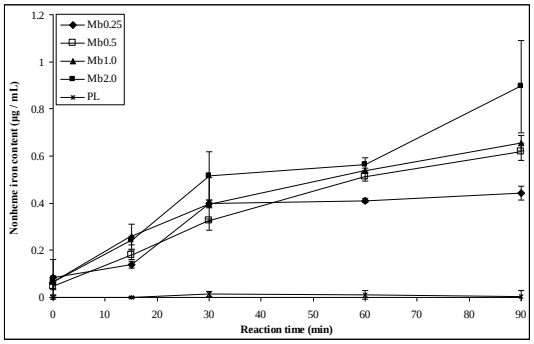
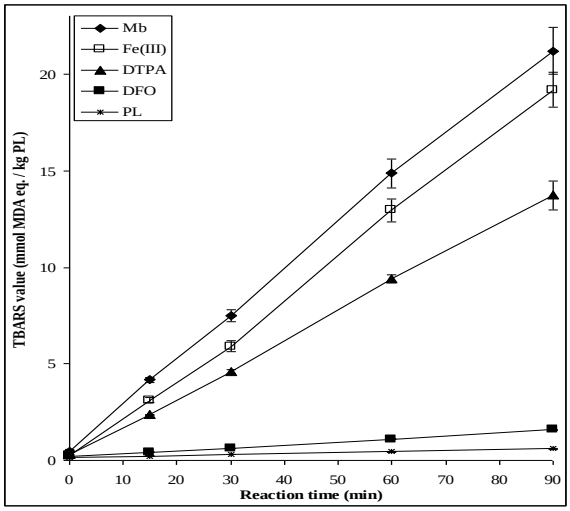


Figure 3. Lipid oxidation potential of metMb treated with desferrioxamine (DFO, 2 mM; final concentration), diethylenetriamine pentaacetic acid (DTPA, 2 mM; final concentration), or ferric chloride (Fe(III), 5 µg/mL; final concentration) in phospholipid liposome model system during incubation at 37 °C for 90 min (TBARS value, mmol malondialdehyde (MDA) equivalents/kg phospholipid (PL)). The final concentrations of metMb in liposome solution were 0.25 (A) and 1.0 (B) mg per mL, respectively. Phospholipid liposome model system with metMb and buffer were used as a control (Mb) and blank control (PL), respectively.

A. 0.25 mg metmyoglobin/mL



B. 1.0 mg metmyoglobin/mL

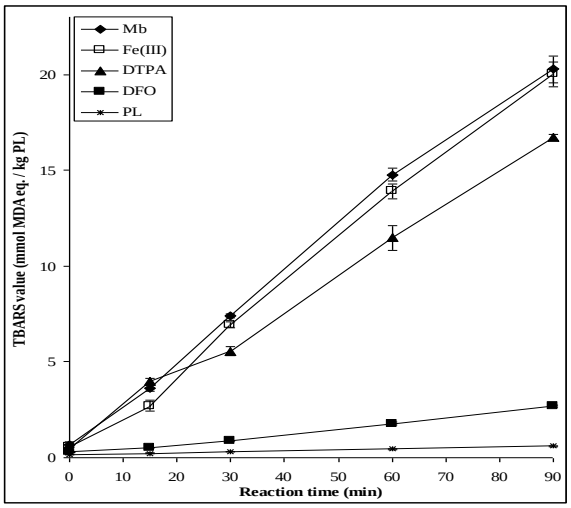


Figure 4. Lipoxygenase-like activity (Unit/mL) of metMb solution treated with none (control), metMb (1 mg/mL; final concentration) desferrioxamine (DFO, 2 mM; final concentration), diethylenetriamine pentaacetic acid (DTPA, 2 mM; final concentration), or ferric chloride (Fe(III), 5 µg/mL; final concentration) in 50 mM acetate buffer, pH 5.6.